

# Multistep resistance generation evaluation

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An abbreviated version of this protocol was published in Science Advances in Jan 2021

A polymeric approach toward resistance-resistant antimicrobial agent with dual-selective mechanisms of action

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## Detailed protocol

- For the first passage, the broth microdilution method for MIC determination of compounds was performed against the bacteria. An overnight starter culture of bacteria in CAMHB was diluted 1000-fold in fresh media, and grown at 37 °C to reach an OD<sub>600</sub> of approximately 0.3.
- This log-phase culture was diluted in fresh media to generate the working solution containing  $5 \times 10^5$  CFU/mL bacteria.
- 100  $\mu$ L of this working solution was transferred into every well of a flat-bottom 96-well plate, except for the first column (which was reserved for CAMHB as negative control) and the first row (reserved for initial compound solutions).
- Compounds were added at specific starting concentrations with a total volume of 200  $\mu$ L working solution into the first row.
- The compounds were then sequentially diluted twofold across 8 wells with working solution. Plates were incubated at 37 °C, shaking at 220 rpm, for 24 h.
- The OD<sub>600</sub> values were measured to determine MIC values of compounds.
- For each subsequent passage, the inoculum for MIC determination was adjusted to a final density of approximately  $5 \times 10^5$  CFU/mL using the contents of a well containing compounds at a sub-inhibitory concentration (at which bacterial growth was observed from the previous passage).
- To measure the MIC of each passage, bacteria were transferred to a new 96-well microtiter plate. Then repeat the step 4-7.
- Resistance was classified as a greater than a 4-fold increase in the initial MIC.

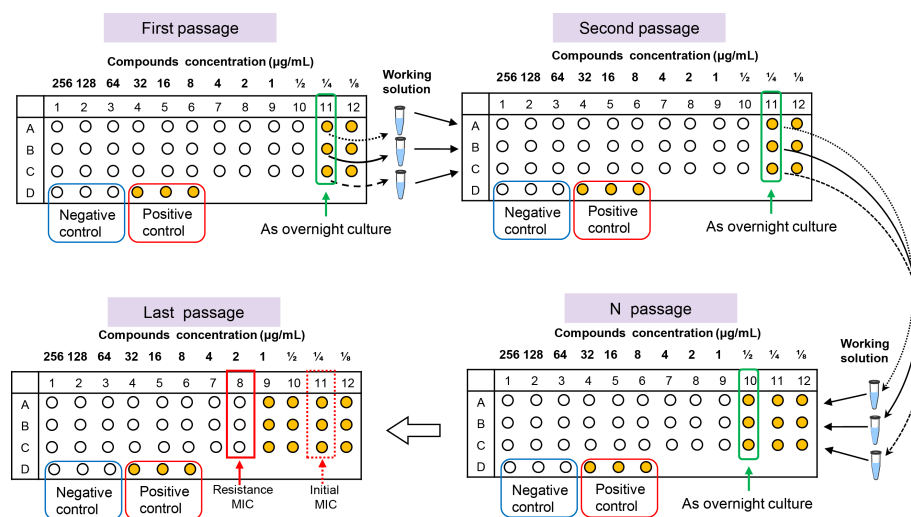


Figure 1. Flow chart for multistep resistance generation experiment.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

- Bai, S. , Wang, J. , Bai, Y. and 冯, 欣. (2021). Multistep resistance generation evaluation. Bio-protocol Preprint. [bio-protocol.org/prep1184](https://doi.org/10.1101/2021.06.22.446917).
- Bai, S., Wang, J., Yang, K., Zhou, C., Xu, Y., Song, J., Gu, Y., Chen, Z., Wang, M., Shoen, C., Andrade, B., Cynamon, M., Zhou, K., Wang, H., Cai, Q., Oldfield, E., Zimmerman, S. C., Bai, Y. and Feng, X.(2021). A polymeric approach toward resistance-resistant antimicrobial agent with dual-selective mechanisms of action. Science Advances 7(5). DOI: [10.1126/sciadv.abc9917](https://doi.org/10.1126/sciadv.abc9917)

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